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The fungal-derived bicyclo[2.2.2]diazaoctane alkaloids are of significant interest to the scientific community for their potent and varied biological activities. Within this large and diverse family of natural products the insecticidal metabolite (+)-brevianamide A is particularly noteworthy for its synthetic intractability and inexplicable biogenesis. Despite five decades of research, this alkaloid has never succumbed to chemical synthesis due to insurmountable issues of reactivity and selectivity associated with all previously explored strategies. We herein report the first chemical synthesis of (+)-brevianamide A (7 steps, 7.2% overall yield, 750 mg scale), which includes a bio-inspired cascade transformation of the linearly fused (−)-dehydrobrevianamide E into the topologically complex bridged-spiro-fused structure of (+)-brevianamide A.

The Diels–Alder cycloaddition is one of the most important reactions in synthetic organic chemistry, allowing the stereoselective construction of six-membered rings through the concerted formation of two new bonds (Figure 1a).[1] The limited appearance of Diels–Alder reactions in biosynthetic pathways has always fascinated chemists.[2-6] The bicyclo[2.2.2]diazaoctane alkaloids, which are a vast group of natural products isolated from various marine and terrestrial fungi, have played a key role in the development of our understanding of biosynthetic Diels–Alder reactions.[7,8] They are also of broader interest to both chemists and biologists alike due to their important biological activities, diverse biosynthetic origins, and synthetically daunting structures. There are two distinct families of bicyclo[2.2.2]diazaoctane alkaloids, the monooxopiperazine-type structures (Figure 1b),

which include the anthelmintic paraherquamides,[9-11] calmodulin-inhibiting malbrancheamides,[12] and neuroprotective chrysogenamides;[13] and the dioxopiperazine-type structures (Figure 1c), which include the cytotoxic stephacidins and notoamides,[14,15] and the insecticidal brevianamides.[16,17]

Brevianamides A (**1**) and B (**2**) were originally isolated by Birch and Wright in 1969 from the fungus *Penicillium brevicompactum*,[16] and were the first known bicyclo[2.2.2]diazaoctane alkaloids (Figure 1c).[7,8] Brevianamide A (**1**) exhibits potent antifeedant activity against the larvae of the insect pests *Spodoptera frugiperda* (fall armyworm) and *Heliothis virescens* (tobacco budworm),[17] and is the major isolated diastereomer (ratio of A to B ≳90:10).[18,19] In 1970, Porter and Sammes proposed that the bicyclo[2.2.2]diazaoctane cores of brevianamides A (**1**) and B (**2**) could be biosynthesised through an intramolecular hetero-Diels–Alder cycloaddition (Figure 1d),[20] a proposal which has been extended to encompass all bicyclo[2.2.2]diazaoctane alkaloids.[7,8] Recently, Diels–Alderase enzymes have been identified in the biosynthetic gene clusters responsible for the malbrancheamide and paraherquamide monooxopiperazine-type alkaloids.[21] However, no Diels–Alderase enzyme has yet been identified for the brevianamides, nor any other dioxopiperazine-type alkaloid.[22,23] Furthermore, despite five decades of research, no chemical synthesis of brevianamide A (**1**) has been achieved, yet several syntheses of the minor diastereomer, brevianamide B (**2**), have been reported (for a full summary, see Supplementary Information, Section 1).[24-32] Despite the similar structures of the two natural products, none of these strategies have been successfully applied to the synthesis of brevianamide A (**1**) due to insurmountable issues of reactivity and selectivity.

Following decades of detailed chemical and biochemical studies, the biosynthetic origins of (+)-brevianamide A (**1**) and B (**2**) still remain unknown. We herein propose a modified biosynthetic hypothesis, which builds upon the pioneering work of several research groups.[7,8] To appreciate the origins of this modified biosynthetic proposal it is important to

first outline key details of the elegant work of Williams and co-workers.[26-29] Their biomimetic synthetic studies have mainly focused upon an early proposed pathway (Figure 2, Pathway 1),[33] which was informed by the seminal work of Birch and Sammes.[20,34-36] The pathway begins with a stereoablative oxidation of (+)-deoxybrevianamide E (**3**) to give achiral azadiene **4**, which then undergoes enantioselective Diels–Alder cycloaddition to give a scalemic mixture of bicyclo[2.2.2]diazaoctane enantiomers, **5** and *ent*-**5**. Both enantiomers then undergo *(R)*-selective indole oxidation and a [1,2]-alkyl shift, so that the major enantiomer (**5**) gives brevianamide A (**1**) and the minor enantiomer (*ent*-**5**) gives brevianamide B (**2**). Williams' synthetic studies, however, revealed that this proposed Diels–Alder reaction (**4** to **5**/*ent*-**5**) actually produces an unwanted diastereomer as the major product.[29] Furthermore, biosynthetic feeding experiments with isotopically labelled **5** failed to show any significant incorporation into (+)-brevianamide A (**1**).[37] Williams, therefore, proposed an alternative biosynthetic pathway (Figure 2, Pathway 2), which begins with a diastereoselective indole oxidation of (+)-deoxybrevianamide E (**3**) to give hydroxyindolenine **6**.[37,38] A stereospecific [1,2]-alkyl shift then gives indoxyl **7**, which undergoes a diketopiperazine oxidation and Diels–Alder cycloaddition to give brevianamides A (**1**) and B (**2**).[37,38] Williams and co-workers reasoned that "[t]he preponderance of **1** over **2** would be due either to the relative activities of two different [Diels–Alderase] enzymes or the affinity of a single enzyme active site for the individual conformers".[37] All efforts to substantiate this second-generation pathway *in vitro*, however, have failed, largely due to the instability of indoxyl **7**.[7] Furthermore, hydroxyindolenine **6** is likely to undergo rapid 5-*exo*-*trig* cyclisation to give the known shunt-metabolite brevianamide E (**8**),[16,34] which is proposed to be a biosynthetic dead-end.[37]

Our modified biosynthetic hypothesis for (+)-brevianamide A (**1**) involves an alternative biosynthetic precursor, (+)-dehydrodeoxybrevianamide E (**9**), which is a known natural product isolated from various *Penicillium* and *Aspergillus* species (Figure 3).[39-41]

Crucially, the diketopiperazine ring in (+)-dehydrodeoxybrevianamide E (**9**) is already at the oxidation level required for a later Diels–Alder reaction. Our pathway involves a point-to-point chirality transfer *via* a sequential diastereoselective indole oxidation, stereospecific [1,2]-alkyl shift, and tautomerisation, to give enantiopure azadiene **10**. Diels–Alder cycloaddition of azadiene **10** would then give brevianamides A (**1**) and B (**2**).[37,38] Although our proposed intermediate **11** is likely to undergo reversible 5-*exo*-*trig* cyclisation to give pentacycle **12**, this is not a known natural product and is therefore unlikely to represent a dead-end (cf. brevianamide E (**8**) in Pathway 2, Figure 2). We herein report the first total synthesis of brevianamide A (**1**), following a strategy inspired by our modified biosynthetic proposal.

Results and Discussion

The synthesis of (+)-dehydrodeoxybrevianamide E (**9**) commenced with phthaloyl protection of commercially available L-tryptophan methyl ester **13** (Figure 4).[42] The crude product from this reaction, ester **14**, was subjected directly to Danishefsky's reverse prenylation conditions using *B*-prenyl-9-BBN to give intermediate **15** in 69% yield over the two steps.[43] Hydrolysis of methyl ester **15** was accompanied by ring opening of the phthaloyl group to give diacid **16**, which presumably explains why a protecting group switch from phthaloyl to *tert*-butyloxycarbonyl or trityl has been undertaken in related synthetic endeavours.[43,44] To avoid these extraneous two steps (deprotection/re-protection) we explored the possibility of an S_N2-type demethylation of methyl ester **15**. While LiI in EtOAc, as reported by Fisher and Trinkle,[45] gave the desired product **17**, we found LiCl in DMF gave a cleaner reaction. Lithium carboxylate **17** was then subjected to a one-pot acyl chloride formation and imine acylation reaction with dehydroproline **18** to give *N*-acyl enamine **19**.[46] Attempts to deprotect the primary amine in **19** using conventional phthaloyl deprotection reagents, such as hydrazine, ethylene diamine, methylamine, hydroxylamine, ethanolamine, and phenylhydrazine resulted in unwanted cleavage of the amide bond. Our search for less

nucleophilic reagents eventually led us to ammonia in methanol,[47] which not only deprotected the primary amine but also resulted in spontaneous cyclisation to give (+)-dehydrodeoxybrevianamide E (**9**) in 49% yield over the three steps from methyl ester **15**. Thus, by developing a new imine acylation reaction and avoiding unnecessary protecting group manipulations, the shortest total synthesis of (+)-dehydrodeoxybrevianamide E (**9**) has been achieved, proceeding in a longest linear sequence of 5 steps, 34% overall yield, and requiring just two chromatographic purifications (cf. previous synthesis: 12 steps, 8% overall yield).[29]

Oxidation of (+)-dehydrodeoxybrevianamide E (**9**) using a variety of oxidants (*e.g.*, dioxiranes,[43] oxaziridines,[23] singlet oxygen,[48] and peroxy acids) gave dehydrobrevianamide E (**12**), alongside diastereomer **20** (Figure 4). Use of *m*-CPBA gave the highest diastereoselectivity (d.r. 64:36, 57% yield), with no appreciable improvement observed at lower temperatures. The stereoselectivity of related biosynthetic indole oxidations is known to be controlled by flavin-dependent monooxygenase enzymes.[8] Synthetic access to both diastereomers **12** and **20**, however, enabled us to prepare both the natural-(+) and unnatural-(−) enantiomers of brevianamides A (**1**) and B (**2**) (*vide infra*). Exposure of dehydrobrevianamide E (**12**) to LiOH in water at ambient temperature for 30 minutes successfully gave (+)-brevianamide A (**1**) and (+)-brevianamide B (**2**) in a combined 63% yield, presumably *via* the domino retro-5-*exo*-*trig*/[1,2]-alkyl shift/Diels–Alder reaction sequence outlined in Figure 3. Keeping the reaction time to a minimum and avoiding elevated temperatures was essential to prevent alkaline hydrolysis of the products, as previously observed by Birch and co-workers.[34] Following purification by column chromatography, which is only the fourth purification in the entire seven-step synthesis, 750 milligrams of (+)-brevianamide A (**1**) and 60 milligrams of (+)-brevianamide B (**2**) were isolated.[49] Chiral-HPLC analysis revealed both products (**1** and **2**) were isolated in a 93:7 enantiomeric ratio, which is not surprising given many of our synthetic intermediates may have undergone

partial racemisation; recrystallisation gave (+)-brevianamide A (**1**) in an enantiomeric ratio of 99:1. The unnatural (−)-enantiomers of brevianamides A (**1**) and B (**2**) were similarly accessed by subjecting the minor diastereomer, compound **20**, to the same reaction conditions (Figure 4).

The Diels–Alder reaction produces (+)-brevianamide A (**1**) and (+)-brevianamide B (**2**) in a 93:7 diastereomeric ratio (Figure 3), which closely matches the ratio observed when they are isolated from *Penicillium brevicompactum* (for further details, see Supplementary Information, Section 2).[18] Thus, we suggest that this Diels–Alder reaction might be a spontaneous process exploited by Nature without direct enzyme participation.[37] This is in contrast to the requirement of Diels–Alderase enzymes in the stereoselective biogenesis of the closely related malbrancheamide and paraherquamide alkaloids.[21] During the review process for this manuscript, Williams, Sherman, Li and co-workers reported their identification of the brevianamide A biosynthetic gene cluster.[50] Their detailed biosynthetic studies, which included targeted gene disruption, heterologous expression, precursor incorporation studies, and *in vitro* biochemical analysis also support a spontaneous, non-enzyme mediated, biosynthetic Diels–Alder reaction.[50]

In summary, 50 years after its discovery by Birch,[16] the first chemical synthesis of brevianamide A (**1**) has been achieved. Key to the success of this synthesis is a bio-inspired one-step cascade transformation of the linearly fused pentacyclic dehydrobrevianamide E (**12**) into the far more complex hexacyclic structure of brevianamide A (**1**). This complexity generating sequence would likely not have been designed from a purely retrosynthetic analysis of the target, demonstrating the unique utility of the biomimetic approach in natural product synthesis.

Data availability

All the characterization data and experimental protocols are provided in this article and its Supplementary Information. Data are also available from the corresponding author upon

reasonable request. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, under deposition numbers CCDC 1918446 (**1**). Copies of the data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/.

Acknowledgements

This work was supported by an EPSRC First Grant (EP/N029542/1) and a Marie Curie Career Integration Grant (631132, POSIN). We would like to thank Mr Tony Herlt for assistance and advice regarding chromatography, and acknowledge SIRCAMS at the University of Edinburgh for mass spectrometry.

Author Contributions

R.C.G., N.J.G. and A.L.L. conceived, designed and carried out the synthetic experiments. G.S.N. performed the crystallographic studies. All authors discussed and co-wrote the manuscript.

Competing Interests

The authors declare no competing interests.

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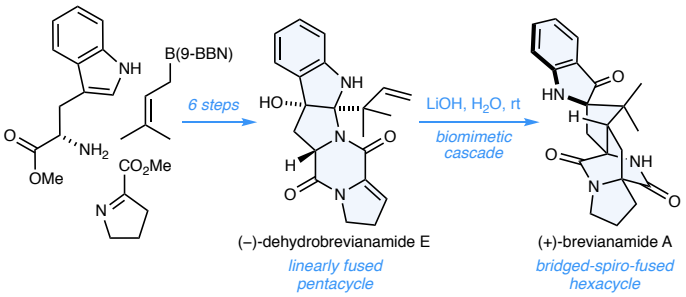
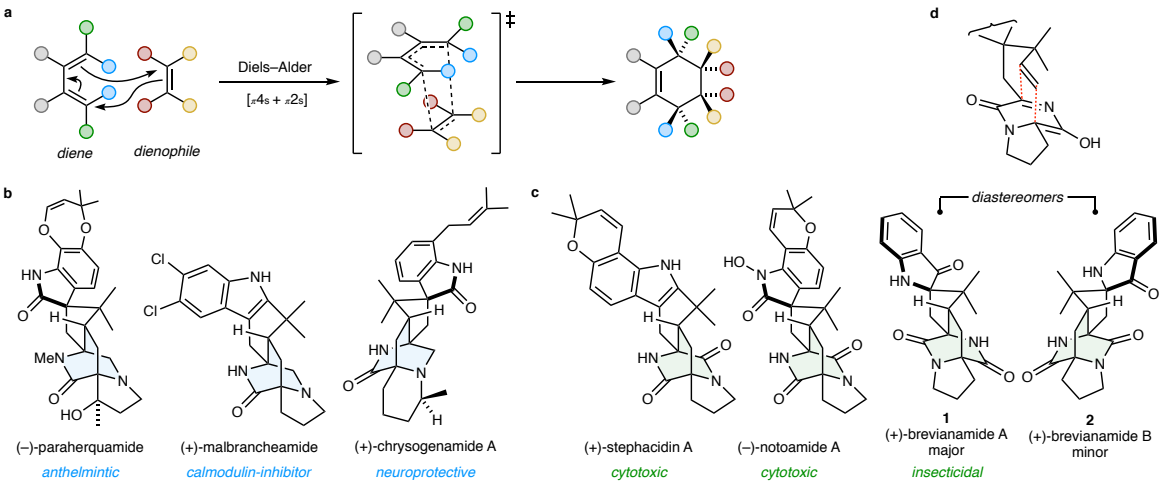
Fig. 1 The Diels–Alder cycloaddition and representative examples of bicyclo[2.2.2]diazaoctane alkaloids, which are proposed to be biosynthesised via intramolecular hetero-Diels–Alder reactions. a, The Diels–Alder cycloaddition. **b**, Representative monooxopiperazine-type bicyclo[2.2.2]diazaoctane alkaloids (bicyclo[2.2.2]diazaoctane cores highlighted in blue). **c**, Representative dioxopiperazine-type bicyclo[2.2.2]diazaoctane alkaloids (bicyclo[2.2.2]diazaoctane cores highlighted in green). **d**, Sammes' proposed biosynthetic hetero-Diels–Alder reaction.

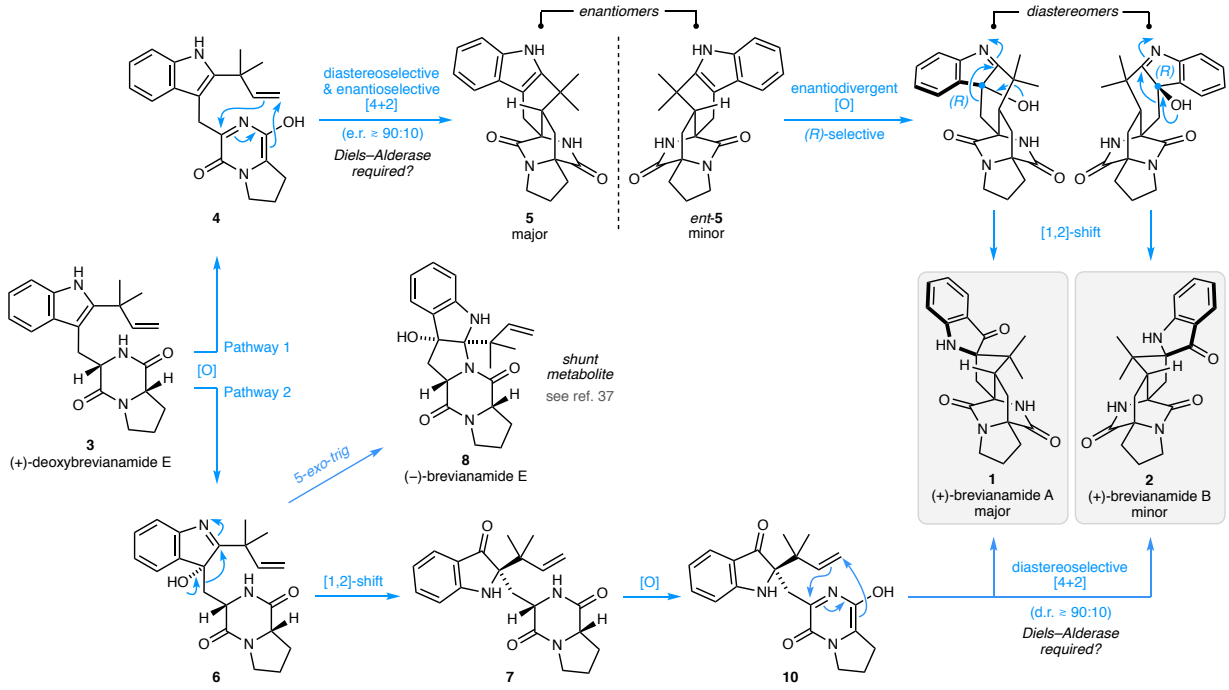
Fig 2 Previous biosynthetic proposals for brevianamides A and B. In 1989, building on the earlier work of Birch and Sammes, Williams and co-workers proposed Pathway 1 as a plausible biosynthetic pathway towards brevianamides A and B.[33] In 1993, following their synthetic and biosynthetic studies, Williams and co-workers proposed an alternative order of biosynthetic transformations, as shown in Pathway 2.[37] Biosynthetic feeding experiments with isotopically labelled **5** and (−)-brevianamide E (**8**), however, show no significant incorporation into **1** or **2**.

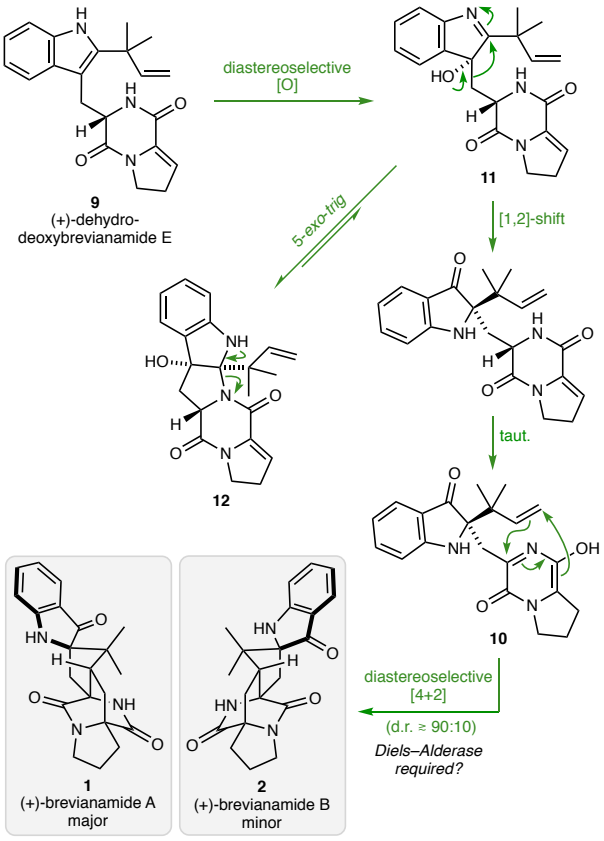
Fig. 3 A modified biosynthetic proposal for brevianamides A and B. The known natural product (+)-dehydrodeoxybrevianamide E (**9**), which has the diketopiperazine ring at the oxidation level required for a later Diels–Alder reaction, is invoked as an alternative biosynthetic precursor towards brevianamide A and B. This avoids the intermediacy of indoxyl **7** (Figure 2, Pathway 2), the instability of which has thwarted previous biomimetic approaches. Although oxidation of (+)-dehydrodeoxybrevianamide E (**9**) is expected to give compound **12**, akin to the oxidative ring closure of **3** to give **8** (Figure 2, Pathway 2), (−)-dehydrobrevianamide E (**12**) is not a known natural product and is therefore less likely to represent a biosynthetic dead-end.

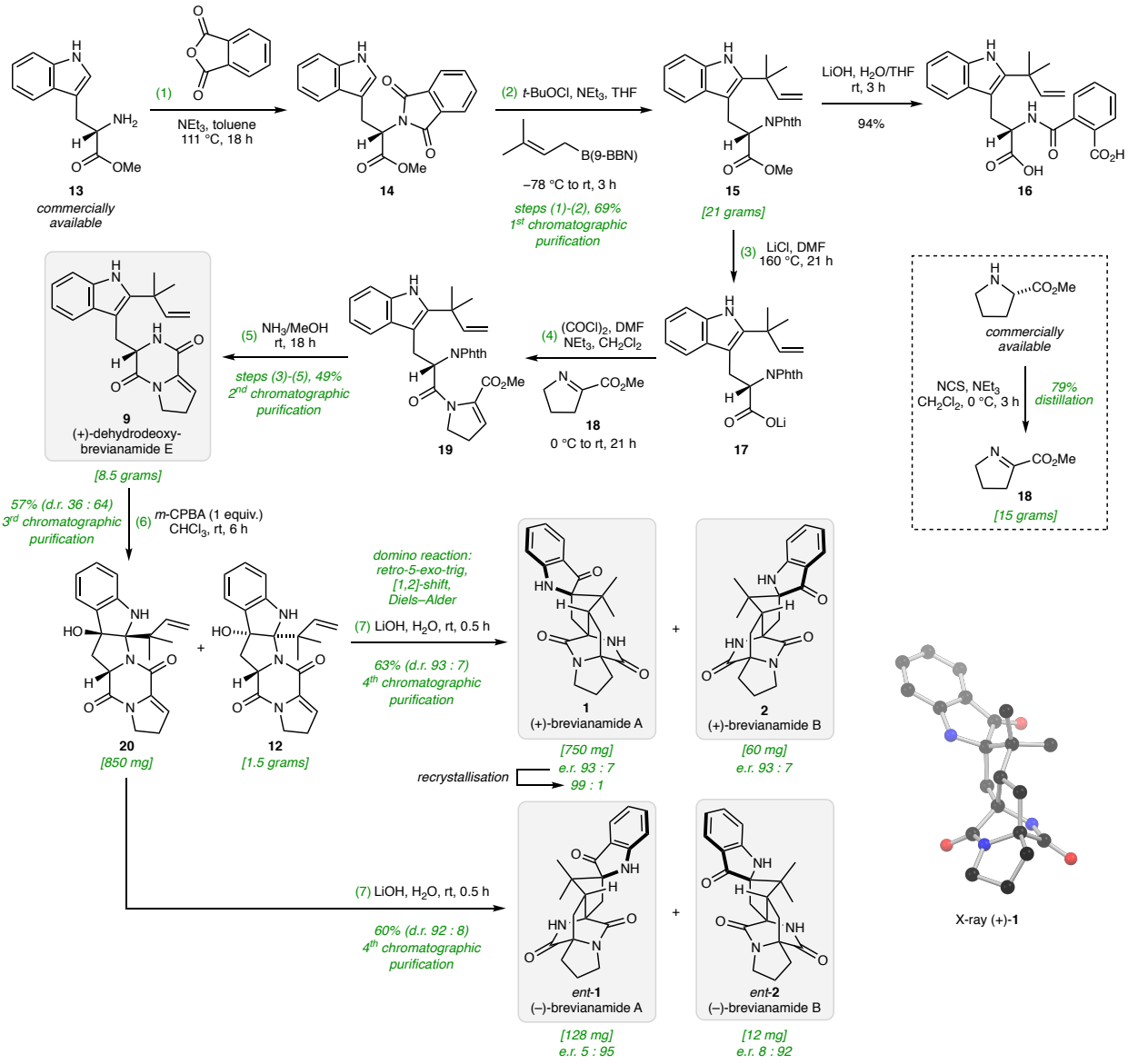
Fig. 4 Total synthesis of brevianamides A and B. The synthesis begins with the shortest reported total synthesis of (+)-dehydrodeoxybrevianamide E (**9**) (5 steps, 34% overall yield, 8.5 gram scale). Oxidation of (+)-dehydrodeoxybrevianamide E (**9**) gives a diastereomeric mixture of dehydrobrevianamide E (**12**) and **20**. Exposure of **12** and **20** to LiOH in water gives the natural and unnatural enantiomers of (+)-brevianamides A (**1**) and B (**2**), respectively. 9-BBN, 9-borabicyclo(3.3.1)nonane; Phth, phthaloyl; DMF, dimethylformamide; NCS, *N*-chlorosuccinimide; *m*-CPBA, *meta*-chloroperoxybenzoic acid.


B(9-BBN)
6 steps
LiOH, H2O, rt
biomimetic cascade
(–)-dehydrobrevianamide E
linearly fused pentacycle
(+)-brevianamide A
bridged-spiro-fused hexacycle


a
diene
dienophile
Diels–Alder
[π4s + π2s]
‡
d
b
(–)-paraherquamide
anthelmintic
(+)-malbrancheamide
calmodulin-inhibitor
(+)-chrysogenamide A
neuroprotective
c
(+)-stephacidin A
cytotoxic
(–)-notoamide A
cytotoxic
diastereomers
1
(+)-brevianamide A
major
insecticidal
2
(+)-brevianamide B
minor


enantiomers
diastereomers
diastereoselective & enantioselective [4+2]
(e.r. ≥ 90:10)
Diels–Alderase required?
enantiodivergent [O]
(R)-selective
[1,2]-shift
4
5
major
ent-5
minor
1
(+)-brevianamide A
major
2
(+)-brevianamide B
minor
3
(+)-deoxybrevianamide E
Pathway 1
[O]
Pathway 2
5-exo-trig
8
(–)-brevianamide E
shunt metabolite
see ref. 37
6
[1,2]-shift
7
[O]
10
diastereoselective [4+2]
(d.r. ≥ 90:10)
Diels–Alderase required?


diastereoselective
[O]
9
(+)-dehydro-
deoxybrevianamide E
11
[1,2]-shift
5-exo-trig
12
taut.
10
diastereoselective
[4+2]
(d.r. ≥ 90:10)
Diels–Alderase
required?
1
(+)-brevianamide A
major
2
(+)-brevianamide B
minor


(1) NEt3, toluene 111 °C, 18 h
(2) t-BuOCl, NEt3, THF
B(9-BBN)
–78 °C to rt, 3 h
LiOH, H2O/THF rt, 3 h
94%
(3) LiCl, DMF 160 °C, 21 h
(4) (COCl)2, DMF NEt3, CH2Cl2
0 °C to rt, 21 h
(5) NH3/MeOH rt, 18 h
(6) m-CPBA (1 equiv.) CHCl3, rt, 6 h
(7) LiOH, H2O, rt, 0.5 h
recrystallisation
NCS, NEt3 CH2Cl2, 0 °C, 3 h
13
commercially available
14
15
[21 grams]
16
steps (1)-(2), 69%
1st chromatographic purification
17
18
0 °C to rt, 21 h
19
steps (3)-(5), 49%
2nd chromatographic purification
9
(+)-dehydrodeoxy-brevianamide E
[8.5 grams]
57% (d.r. 36 : 64)
3rd chromatographic purification
20
[850 mg]
12
[1.5 grams]
domino reaction: retro-5-exo-trig, [1,2]-shift, Diels–Alder
63% (d.r. 93 : 7)
4th chromatographic purification
1
(+)-brevianamide A
[750 mg]
e.r. 93 : 7
99 : 1
2
(+)-brevianamide B
[60 mg]
e.r. 93 : 7
60% (d.r. 92 : 8)
4th chromatographic purification
ent-1
(–)-brevianamide A
[128 mg]
e.r. 5 : 95
ent-2
(–)-brevianamide B
[12 mg]
e.r. 8 : 92
79% distillation
18
[15 grams]
X-ray (+)-1